EXPERIMENT K-6-08

BIOCHEMICAL AND HISTOCHEMICAL OBSERVATIONS OF VASTUS MEDIALIS

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INTRODUCTION

Muscles of the hindlimb in the rat have been used to demonstrate the effects of unloading in weightlessness and in animal models used to mimic weightlessness. This report deals with the vastus medialis (VM). Samples were obtained from rats exposed to weightlessness for 12 days in Cosmos 1887 (Experiment K-6-08, coordinated by Dr. V.S. Oganov).

The VM in the rat is chiefly composed of fast twitch fibers, comparably divided between oxidative glycolytic and glycolytic types (Ariano, et al., 1973). In many respects it is similar to the extensor digitorum longus (EDL), chiefly fast twitch fibers (97%), oxidative glycolytic and glycolytic (59 and 38%, respectively) and a few (3%) slow twitch fibers (Ariano, et al., 1973). The VM and EDL are not load bearing muscles and, in addition to morphological similarity, it is reasonable to assume that there is some functional and metabolic similarity. We obtained the VM because of its availability and it afforded us an opportunity to compare data with the EDL which has been used in both microgravity flights (Ilyina-Kakueva, et al., 1976, Oganov and Potapov, 1976, Castleman and Chui, 1978, and Steffen and Musacchia, 1986) and earthside model experiments (Musacchia, et al, 1980, and Fitts, et al, 1986).

The EDL is recognized as being relatively unaffected by exposure to weightlessness for periods of seven (SL-3) to 22 (Cosmos 605) days (Steffen and Musacchia, 1986, and Ilyina-Kakueva, et al, 1976). Also, there is limited disuse atrophy in response to unloading in earthside laboratory experiments using both tail suspension (Jaspers and Tischler, 1986) and whole body suspension (Musacchia, et al., 1980) models.

The principal objectives of the present study were to ascertain if the VM responded to 12 days of microgravity exposure. Three approaches were used: (a) a histochemical evaluation of cellular morphology (fibers and capillaries), (b) an assessment of biochemical composition (protein, RNA and DNA concentrations) and (c) an estimation of metabolic activities and capacities (oxidative and glycolytic metabolism).

MATERIALS AND METHODS

Samples of muscles were obtained from five rats exposed to weightlessness for 12 days (F), basal control rats (B), vivarium control rats (V) and synchronous control rats treated comparably to rats exposed to weightlessness (S). Muscles were frozen in liquid nitrogen and shipped on dry ice to the University of Louisville. Samples from the belly of two muscles from rats in each group were examined histochemically for morphometric characteristics as in a previous report (Musacchia, et al., 1987). Frozen sections were stained for ATPase activity, muscle fibers and capillaries were differentiated. Fiber area and density measurements were made and capillary distribution was assessed. The remaining muscle samples were lyophilized, weighed and powdered with a Wiley Mill. Aliquots were used for protein (mg/mg dry wt.) RNA and DNA concentration determinations (ug/mg dry wt) as previously described (Steffen and Musacchia, 1986), for lactate dehydrogenase (LDH) (Pesce, et al., 1964), citrate synthase (CS) activities (μ moles/min/gm) (Srere, 1969), and lipoprotein lipase (LPL) activities (nmoles FA liberated) (Lithrell and Boberg, 1978).

RESULTS AND DISCUSSION

The principal objective of this study was to ascertain if the vastus medialis responded to 12 days of microgravity exposure. The loss in muscle mass (Table 1) is greatest, -43%, when comparing F vs B, and least, -13%, when comparing F vs. V. Taken at face value these differences may be misleading, due to the variability of the muscle weight in the basal group these muscle mass losses may be exaggerated. In terms of percent water, there were no differences between the flight and

the control groups. In spite of the limited sample, we conclude that muscle mass changes in the VM are not significant.

Comparing the VM from 12 day microgravity exposed rats and the EDL from seven days of microgravity exposured rats, there appears to be much similarity. The EDL lost about 10% mass and this was reflected in a comparison with vivarium weight matched controls (Steffen and Musacchia 1986). This figure compares favorably with the present 13% loss if one compares VM from flight subjects with vivarium controls.

The muscle specimens were found to be almost completely composed of type II fast twitch fibers. In the flight rats examined, there were significant reductions in fiber area (μM^2), i.e., about 30% when compared to basal subjects (Table 2). The fiber area in vivarium controls was comparable to that in flight subjects, however the data from synchronous controls was not uniform and did not provide a basis for comparison. Fiber density measurements (cells/mm^2) were inversely related to the area measurements. The limited morphological responses in the VM are comparable to the EDL after seven days of flight (Musacchia, et al., 1987).

Protein concentrations in flight, basal and synchronous subjects were comparable (0.7 to 0.8 mg/mg dry wt) (Table 3). However, the vivarium controls exhibited higher values (0.88 ± .06 mg/mg dry wt.). RNA concentrations in flight subjects (5.5 ± .1 µg/mg dry wt) were significantly reduced below basal controls (6.0 ± .1 µg/mg dry wt). RNA concentrations in flight subjects were not different from either vivarium (5.5 ± .1 µg/mg dry wt) or synchronous (5.1 ± .6 µg/mg dry wt) controls. DNA concentrations in flight, vivarium and basal subjects were similar (13 to 14 µg/mg dry wt), however, the synchronous controls exhibited markedly higher values (16.8 ± .9 µg dry wt). The biochemical profiles suggest that changes in the VM due to weightlessness were minimal. In this respect the VM is similar to the EDL, which was examined by us following the SL-3 mission (Steffen and Musacchia 1986).

The level of LDH activity (>2100) is characteristic of fast twitch highly glycolytic (type II B) fibers (Figure 1). Conversely, the oxidative capacity (circa 10 µ moles/min/gm) as measured by CS activity was low and characteristic of fast twitch muscle (Figure 2). These results are supported by the histochemical data.

LPL measurements indicate that enzyme activity in flight rats is lower than the vivarium and basal control roups (Figure 3). This could suggest that VM from flight rats had a reduced capacity to utilize stored triglycerides for energy production. However, the lack of difference between flight and synchronous control rats indicates that the previous deduction must be considered with caution.

CONCLUSIONS

Although some of the morphological parameters suggest a small degree of atrophy in the vastus medialis, the biochemical analyses (protein, RNA and DNA) suggest that these may be minimal and functionally nonsignificant. The relatively similar CS and LDH activities of VM from F and various control groups, as well as the lack of difference in LPL activity between F and S rats, suggests that there is little or no effect on the oxidative or glycolytic function of this muscle. Since the VM is chiefly a mixed fast twitch muscle, these metabolic indices of energy production are relatively unchanged. The results of VM studies are in agreement with our previous observations of another type II fast twitch muscle, the EDL, from SL-3 rats which did not respond markedly to weightlessness and whole body suspension.

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TABLE 1

RAT, VASTUS MEDIALIS AND BODY WEIGHTS

Groups	Body Wt (gm)	Muscle Wt (mg)	Muscle Wt/B Wt	%Water
Flight	303 ±2.4	334 ± 41.1	1.1 ± 0.14	73.9± 2.16
Controls				
Basal	316 ± 8.3	586 ± 85.0	1.9 ± 0.28	74.2 ± 0.64
Vivarium	342 ± 7.7	386 ± 31.0	1.1 ± 0.08	75.5 ± 0.72
Synchronous	349 ± 5.8	427 ± 19.4	1.2 ± 0.04	75.8 ± 0.26

TABLE 2

RAT, VASTUS MEDIALIS, MORPHOMETRIC MEASUREMENTS

Groups	Rat Number	Fast Twitch Fibers Cross Sec * Area (μm^2)	Fast Twitch Fibers Density** (Cells /mm^2)	Capillary *** Density (cap/mm^2)
Flight	8	3889	249	565
	10	3852	253	725
Controls				
Basal	7	5536	190	374
	8	5201	202	409
Vivarium	6	3469	239	554
	9	3940	247	634
Synchronous	7	3562	156	390
	9	3962	266	673

Type II * number of cell areas measured; 40 or more
** number of cells counted; 70 to 100
*** number of capillaries counted; 300 to 700

TABLE 3

RATS, VASTUS MEDIALIS, BIOCHEMICAL OBSERVATIONS

Groups	Protein (mg/mg Dry Wt)	RNA (µg/mg Dry Wt)	DNA (µg/mg Dry Wt)
Flight	0.70 ± 0.07	5.5 ± 0.1	14.1 ± 0.6
Controls			
Basal	0.73 ± 0.66*	6.0 ± 0.1	13.9 ± 0.4
Vivarium	0.88 ± 0.06	5.5 ± 0.1	12.9 ± 1.4
Synchronous	0.81± 0.0o	5.1 ± 0.6	16.8 ± 0.9

*Mean ± S.E. N = 5

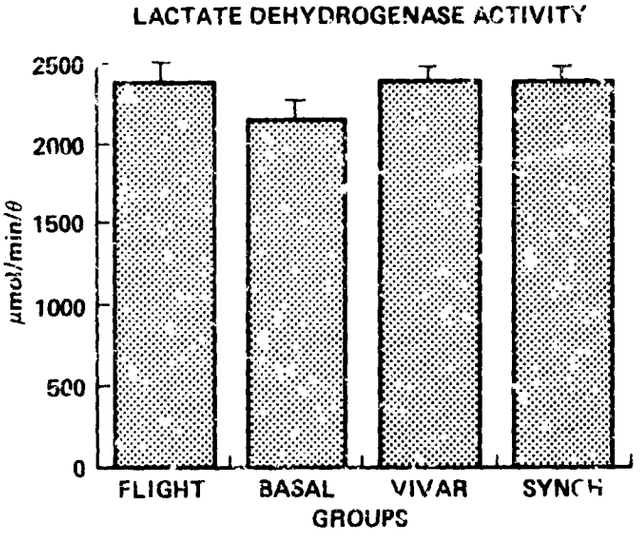

Figure 1. Lactate dehydrogenase activity of vastus medialis from rats: flight and control groups (5 in each group; mean and SEM).

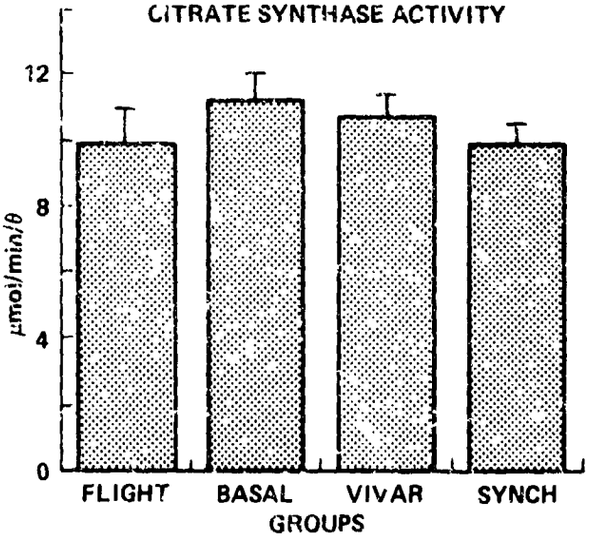

Figure 2. Citrate synthase activity of vastus medialis from rats: flight and control groups (5 in each group; mean and SEM).

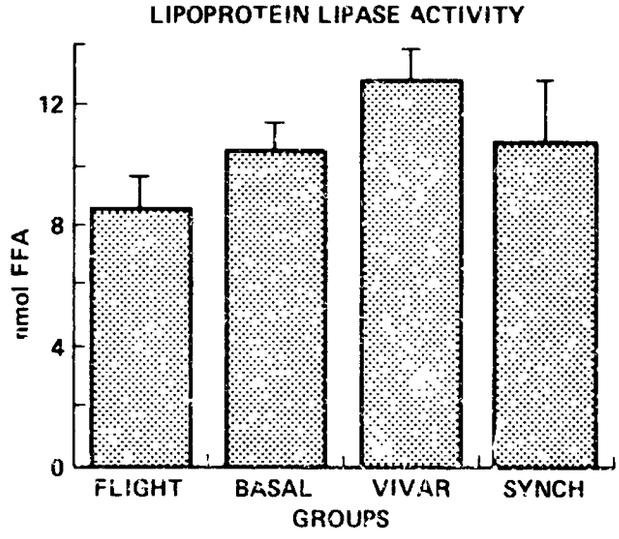

Figure 3. Lipoprotein lipase activity of vastus medialis from rats: flight and control groups (5 in each group; mean and SEM).